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TITLE: Control of Atherosclerosis Regression by PRMT2 in Diabetes

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14. ABSTRACT
Diabetics have more heart disease than their non-diabetic counterparts, even though drugs like statins are equally effective in both groups at lowering the blood levels of harmful cholesterol. We have identified an enzyme called PRMT2, which regulates the abundance of a cellular cholesterol transporter that helps to prevent cells from accumulating in arteries and forming a plaque. We have shown that the level of PRMT2, while high in healthy cells, is very low in cells from diabetics when blood sugar levels are elevated. Because PRTM2 isn't around in cells under diabetic conditions, we predict that more cells accumulate in the artery, thus allowing the plaque to grow and exacerbating heart disease in diabetics. To test this, we will determine what happens to the growth of a plaque in an artery when we eliminate PRMT2 with and without diabetes in mouse models of heart disease. We expect that plaques will grow larger in the absence of PRMT2. To better understand how PRMT2 suppresses plaque growth, we will also identify proteins that are modified by PRMT2 in cells from the plaque and determine if these proteins participate in plaque formation. Given that we also don't understand why PRMT2 levels decrease in diabetes, we will identify cellular proteins that regulate PRMT2 levels. That knowledge might enable us to develop ways to restore the normal level of PRMT2 in diabetes, and prevent the cells from contributing to plaque formation, and reduce heart attacks.

15. SUBJECT TERMS
atherosclerosis regression, diabetes, PRMT2,

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1. INTRODUCTION:

High plasma cholesterol and diabetes are major risk factors for atherosclerosis. People with diabetes are 2-4 times more likely to suffer from coronary heart disease. Understanding the factors that are altered as a result of impaired glucose homeostasis is a significant area of cardiovascular research. Work from our labs has shown that regression of atherosclerosis is mediated in part by the Liver X Receptor (LXR) through the induction of genes involved in cholesterol efflux. We also found that regression of atherosclerosis is impaired in the context of diabetes, suggesting that changes in glucose levels reduce the expression of LXR target genes like the cholesterol transporter protein ABCA1, and that this molecular mechanism might account for the increased rate of atherosclerosis in diabetics. We identified the protein arginine methyltransferase 2 (PRMT2), cellular levels of which are reduced in high *versus* normal glucose, as a factor that mediates this effect. The goals of this grant are to (i) define the role of PRMT2 in atherosclerosis regression in diabetes in mouse models, (ii) elucidate the mechanism of how PRMT2 controls gene expression by identifying PRMT2 substrates, and (iii) determine how the PRMT2 gene itself is regulated in macrophages in response to hyperglycemia. Achieving these goals will enable us to develop new therapeutic strategies to restore normal PRMT2 expression in diabetics and thereby promote the regression of atherosclerosis in diabetes.

2. KEYWORDS:

Atherosclerosis regression, diabetes, PRMT2

3. ACCOMPLISHMENTS:

The major goals of the project are:

1) Determine the role PRMT2 plays in the impaired regression of atherosclerosis in diabetes

2) Determine the substrates of PRMT2 in macrophages in normal and high glucose that affect LXR transcriptional activity

3) Determine the molecular regulation of PRMT2 and exploit this information to restore macrophage PRMT2 expression in diabetes to promote the regression of atherosclerotic plaques.

Specific Aim 1: To determine the role PRMT2 plays in the impaired regression of atherosclerosis in diabetes

Major Task 1: Bone Marrow transplant from PRMT2-/- into Reversa mice and regression study -/+ diabetes

Subtask 1: ACURO review and approval; completed for Dr. Garabedian; pending for Dr. Fisher.

Subtask 2: Generating mice for the bone marrow transplant of WT and PRMT2-/- mice into Reversa mice for regression study under normal and diabetic conditions:

It has taken us this year to re derive and generate a cohort of $PRMT2^{-/-}$ mice and litter mate controls for the bone marrow transplant into Reversa mice s to examine regression of atherosclerosis pending AUCRO approval (Fig 1).

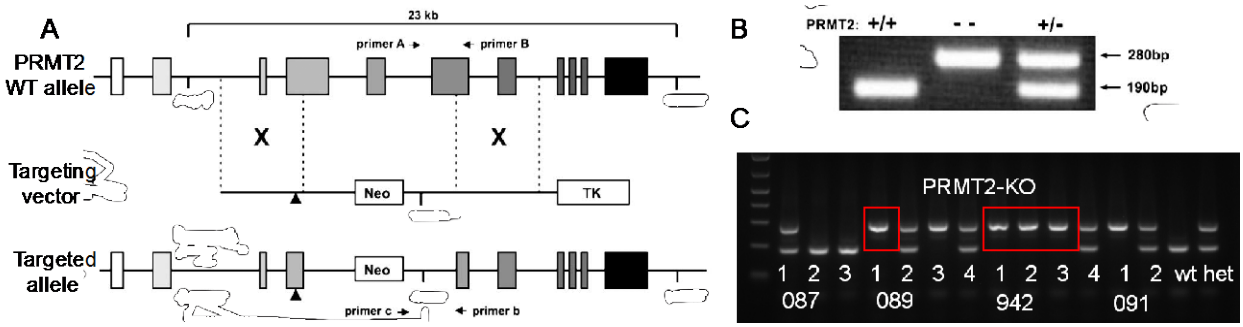
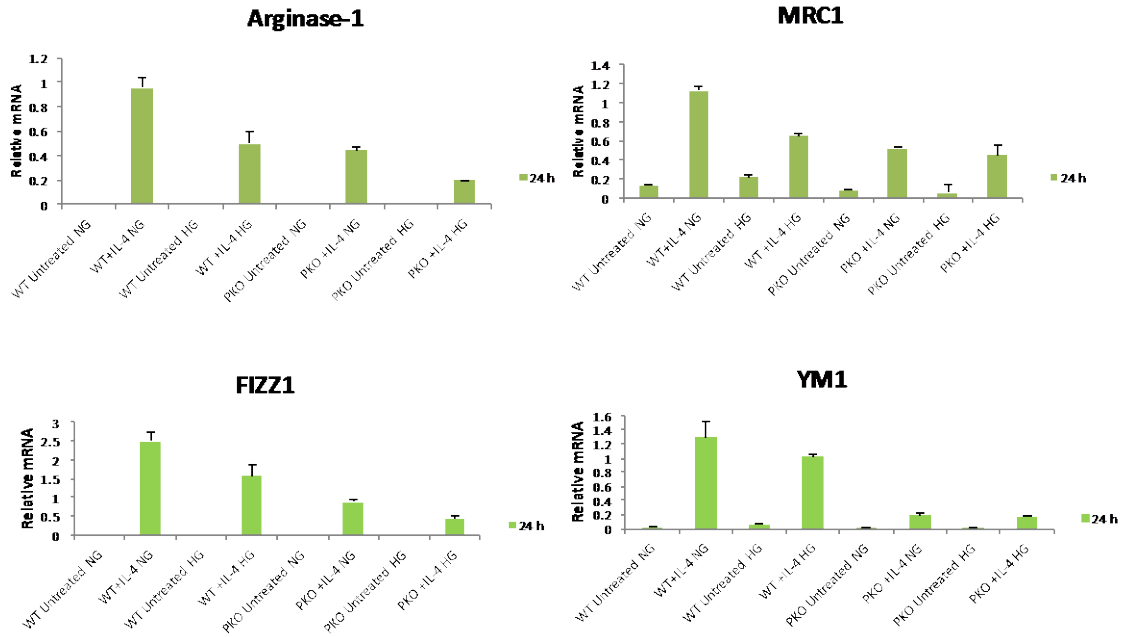


Figure 1 PRMT2$^{-/-}$ mice. (A) Targeted disruption of the PRMT2 locus. The targeting vector was constructed to replace a portion of exon 4, 6 and all of exon 5 with a NeoR gene in an antisense orientation. Point mutation for generating a stop codon (G119stop) is shown as a closed triangle. (B) PRMT2 genotyping by PCR. Combined PCR reaction with sense primer (primer A at exon 4) and two antisense primers (primer B at exon 5; primer C at NeoR gene) were used to detect the wild-type allele (190 bp) or mutant allele (280 bp), respectively. Primer positions are indicated in panel A. (C) Genotyping PRMT2$^{-/-}$ colony. Tail DNA from mice within the NYU Medical Center PRMT2 colony were genotyped as above. Mice within the red rectangles are PRMT2$^{-/-}$ mice.

As a prelude to the *in vivo* regression studies, we have generated bone marrow derived macrophages and examined the effect of PRMT2-deficency on the ability of macrophages to be polarized to M2 phenotype by interleukin 4 (IL4), under normal glucose and diabetes relevant high glucose. Our preliminary studies indicated that PRMT2$^{-/-}$ macrophages are less capable of polarizing to an M2 phenotype as determined by the expression of M2 specified markers, Arginase 1, Mannose receptor 1 (MRC1), FIZZ1, and YIM1. This suggests that the PRMT2 deficient macrophages are less capable of adopting an M2 phenotype to resolve inflammation and this would be predicted to inhibit atherosclerosis regression i*n vivo.*



Major Task 2: Analysis of aortic arches and roots from regression cohort -/+ PRMT2, -/+

Figure 2: PRMT2-deficient macrophages display reduced M2 polarizing capacity. Bone marrow derived macrophages (BMDMs) from WT or PRMT2$^{-/-}$ (PKO) mice were cultured in normal glucose (NG) or high glucose (HG) and either left untreated (UN) or treated with IL4 for 24 h to induce M2 polarization, and mRNA expression of M2 markers Arginase 1, MRC1, FIZZ1 and YM1 determined by qPCR relative the HRPT housekeeping gene.

diabetes;

Not yet started.

Specific Aim 2: To determine the substrates of PRMT2 in macrophages in normal and high glucose that affects LXR transcriptional activity.

Major Task 1: Conduct studies to assess the PRMT2 substrate capture by immunoprecipitation using asymmetric arginine dimethylation antibody

We are optimizing the immunoprecipitation of dimethylated arginine substrates for wild type and PRMT2$^{-/-}$ bone marrow derived macrophages as a lead up to the mass spec studies.

Specific Aim 3: To determine the molecular regulation of PRMT2 and exploit this information to restore macrophage PRTM2 expression in diabetes to promote atherosclerotic plaque regression.

Major Task 1: Mapping PRMT2 cis regulatory elements by reporter gene assays based on ENCODE data;

Subtask 1: Test PRMT2 promoter luciferase construct for glucose regulated expression.

We have tested whether the PRMT2 promoter, is sensitive to changes in glucose using the PRMT2 promoter linked to a luciferase reporter gene and we did not see a change in activity, suggesting the promoter alone is not sufficient to confer glucose regulation on PRMT2 expression.

- **What opportunities for training and professional development did the project provide?**

Nothing to Report."

- **How were the results disseminated to communities of interest?**

We will disseminate our findings through publications in peer reviewed journals, although at this point we have nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals and objectives?**

Our next goals are to perform the bone marrow transplant from wild type and PRMT2-/- mice into LDLR-/- recipients, and examine the effect of PRMT2 on atherosclerosis regression as a function of diabetes (pending AUCRO approval, which is immanent). We will also perform proteomics on the PRTM2 asymmetrically dimethylated substrates using mass spec from WT and PRMT2-/- bone marrow derived macrophages and bone marrow derived macrophages cultured in normal vs high glucose. We will also continue our efforts to map the regulatory elements in PRMT2 responsive to high vs normal glucose.

4. IMPACT:

Our work will determine the impact of PRMT2 in atherosclerosis upon diabetes. We also predict that our proteomic analyses of PRMT2 substrates will be useful in not only elucidating the mechanism of PRMT2 function in macrophages, but also for identifying possible interventions and biomarkers. Finally, understanding the regulation of PRMT2 will enable us to develop ways to restore the normal level of PRMT2 in diabetes, and prevent the cells from

contributing to plaque formation.

What was the impact on technology transfer?

None at this point

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What was the impact on society beyond science and technology?

None at this point

5. CHANGES/PROBLEMS:

We will modify the mouse model of regression away from the Reversa model, which is cumbersome, to a more streamlined model of regression that includes the same LDLR$^{-/-}$ atherogenic background as the Revera model, and uses an anti-sense oligonucleotide (ASO) against the enzyme MTP to lower the lipids to promote regression. This is the same way the Reversa model works to lower cholesterol and is technically simpler to perform. Figure 3 shows the revised model of regression we will employ.

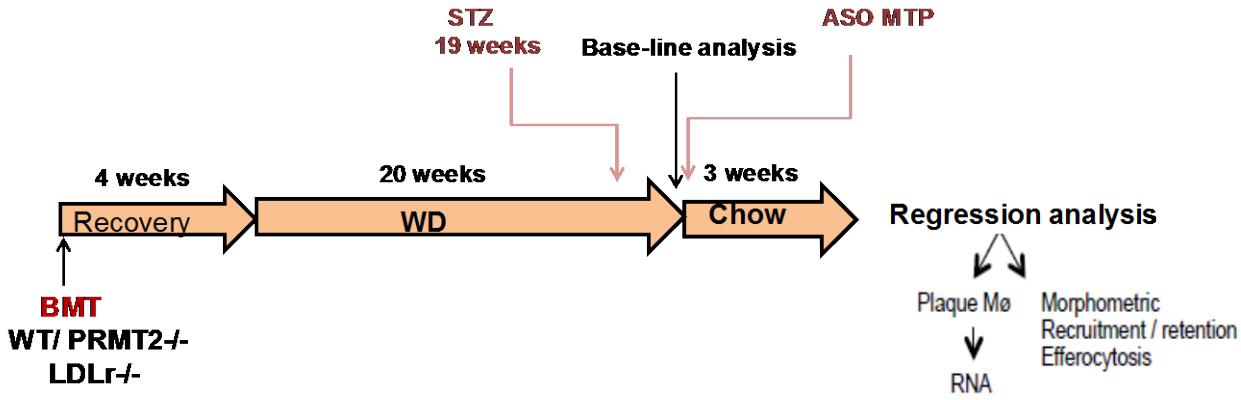

Figure 3. Revised regression model. LDLR-/- mice will be transplanted with bone marrow from PRMT2-/- or littermate wild type controls, let to recover for 4 weeks, and fed a western diet (20% fat, 0/3% cholesterol) for 20 weeks. At 19 weeks half the mice will be made diabetic with STZ treatment. Baseline mice will be harvested. The remaining mice will be treated with an ASO against MTP to reduce lipid secretion from the liver and switched to a chow diet to promote regression. After three weeks, the mice will be analyzed for plaque macrophage content and for macrophage recruitment and retention. Single cell RNA seq on macrophages dissociated from the plaque will also be performed.

6. PRODUCTS:

Nothing to Report

7: PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

PIs

Name: Michael Garabedian, PhD.

Project Role: PI.

Nearest person month worked: 3

Contribution to Project:

Dr. Garabedian helped design and analyze the experiments involving the identification of the PRMT2 substrates and determining the regulation of PRMT2 expression by high glucose.

Funding Support: CDMRP/NIH

Name: Edward Fisher, MD, PhD
Project Role: Partner PI.
Nearest person month worked: 2
Contribution to Project:
Dr. Fisher helped design experiments involving the role of PRMT2 in the regression of atherosclerosis and diabetes.
Funding Support: CDMRP/NIH

Post docs
Prashanth Thevkar Nagesh, PhD
Project Role: Post doc
Nearest person month worked: 9
Contribution to Project:
Dr. Nagesh is performing the experiments involving the PRMT2 substrates identification and the PRMT2 gene regulation studies.
Funding Support: CDMRP/NIH

Beyza Vurusaner Aktas, PhD
Project Role: Post doc
Nearest person month worked: 9
Contribution to Project:
Dr. Aktas is performing the experiments involving the impact of the loss of PRMT2 expression in the regression of atherosclerosis in diabetes.
Funding Support: CDMRP/NIH

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Fisher, Edward A.

Recently Funded

Title:	Macrophage Dysfunction in Obesity, Diabetes and Atherosclerosis
Time Commitment:	3.0 Cal Months
Supporting Agency:	NHLBI
Grants Officer:	John Diggs
Performance Period:	05/01/2017 – 04/30/2022
Level of Funding:	$1,433,608 (Total first year costs of entire Program Project; $446,759 for Dr. Fisher)
Project Goals:	Dr. Fisher is PI of Project 1, which looks at the effects of hyperglycemia on macrophage kinetics in atherosclerotic plaques.
Specific Aims:	Aim 1. To determine the kinetic bases for the increased content of plaque macrophages in diabetic atherosclerotic mice after lipid reduction and to identify key factors/pathways regulated by diabetes and insulin resistance (IR).

	Aim 2. To determine the role of netrin1 on macrophage content and inflammation in the impaired regression of diabetic plaques. Aim 3. To determine the molecular regulation of ACSL1, a major pro-inflammatory factor in macrophages, and its role in atherosclerosis regression in diabetes.
Overlap:	None

Title:	Stress and Atherosclerotic Plaque Macrophages – A Systems Biology Approach – Project 1 Subcontract with ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI
Time Commitment:	0.6 Cal Months
Supporting Agency:	NHLBI
Grants Officer:	Jennifer J Cho
Performance Period:	03/17/2017 – 02/28/2022
Level of Funding:	$250,000 (Co-I with Dr. Kathryn Moore)
Project Goals:	This sub-project focuses on the kinetics of the macrophage population in atherosclerotic plaques in a mouse model of psychological stress.
Overlap:	None

Recently Completed

Title:	Regulation and Function of AKAP12A in the Vessel Wall Subcontract with UNIVERSITY OF ROCHESTER
Time Commitment:	0.96 Cal Months
Supporting Agency:	NHLBI
Grants Officer:	Michelle Olive
Performance Period:	08/01/2013 – 03/31/2017
Level of Funding:	$77,025 for Dr. Fisher (MPI grant with Dr. Joseph Miano)
Project Goals:	The focus of this grant is to investigate the role of the factor AKAP12A in vascular smooth muscle cell differentiation.
Specific Aims:	Specific Aim #1: To elucidate transcriptional/post-transcriptional regulatory control of Akap12a Specific Aim #2: To elucidate the role of AKAP12A in pathological vascular disease Specific Aim #3: To define the AKAP12A-regulated CREBome in VSMC
Overlap:	None

Title:	Theranostic HDL nanoparticles for inflammatory macrophages in atherosclerosis Subcontract ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI
Time Commitment:	0.24 Cal Months
Supporting Agency:	NIBIB
Grants Officer:	Christina Liu
Performance Period:	8/1/13-7/31/17

Level of Funding:	$59,042 to Dr. Fisher (for subcontract on parent grant, Dr. Zahi Fayad, PI)
Project Goals:	The overall project is to use nanoparticles to image mouse atherosclerotic plaques. Dr. Fisher's aims are to exploit the properties of HDL to deliver imaging agents to plaques and to develop HDL as a molecular imaging tool.
Specific Aims:	Aim 1: To create libraries of HDL-like nanoparticles by microfluidics for targeting, visualizing and treating inflammatory macrophages in atherosclerosis. Aim 2: To test the specificity, targeting efficacy and therapeutic potential of HDL nanoparticles for different monocyte and macrophage subpopulations. Aim 3: To conduct MR molecular imaging of inflammatory macrophages in atherosclerotic mice with theranostic HDL nanoparticles. Aim 4: To conduct a therapy study where drug loaded HDL nanoparticles are applied to atherosclerotic mice.
Overlap:	None

What other organizations were involved as partners?

Nothing to Report

8 SPECIAL REPORTING REQUIREMENTS: COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.

An independent report will be submitted by Dr. Garabedian, the contact PI.

9 APPENDICES: none